Gram Staining

Prepare smears using aseptic technique. Several loopful of your sample will be placed first placed on the slide from your broth. This sample is very dilute so you will need to do this MANY times. Remember to sterilize the loop and use proper aseptic technique to transfer your sample.

After air drying and heat fixing the Gram staining procedure is followed.

**GRAM STAINING PROCEDURE:**
1. **Cover with CRYSTAL VIOLET** for 20 seconds. (PRIMARY STAIN)
2. Gently rinse off the stain with water and shake off the excess.
3. **Cover with GRAM'S IODINE** for one minute. (MORDANT)
4. Pour off the Gram's iodine.
5. Run 95% ETHYL ALCOHOL down the slide until the solvent runs clear (about 10-20 seconds). **THIS STEP IS CRITICAL! THICK SMEARS REQUIRE MORE TIME THAN THIN ONES.** (DECOLORIZING AGENT)
6. Rinse with water to stop the action of the alcohol.
7. **Cover with SAFRANIN** for 20 seconds. (COUNTER STAIN)
8. Gently rinse off the stain with water. Blot with bibulous paper and clean off the bottom of the slide with 95% alcohol.
9. View your slide under the scope and loop for your bacteria.

10. **Do not forget to properly clean the oil off of the oil immersion lense!!!!**

**HELPFUL SUGGESTIONS:**
- a) DO NOT make your smears too thick!
- b) Be very careful when you decolorize.